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Running title: PCR-RFLP for detecting *B. tabaci* diversity

Abstract

Bemisia tabaci Gennadius is a major plant pest in many agricultural systems worldwide. It is a complex of cryptic species that differ in many ecological respects, including damage-causing potential. Monitoring the genetic composition of *B. tabaci* populations in the field is, therefore, essential for achieving effective control. Sequencing is costly and time-consuming, thus, efficient diagnostic tools must be developed to perform epidemiological studies involving hundreds of individuals. Here, we describe a PCR-RFLP method for identifying all the putative species comprising the Africa/Middle East/Asia Minor group, including those that are the most devastating crop pests. Moreover, intra-specific diversity associated with specific symbiotic bacteria known to manipulate insect host phenotypes can also be detected. Additionally, this method discriminates between the Africa/Middle East/Asia Minor and the Sub-Saharan African groups, which are sympatric in some areas. This simple, reliable and cost-effective diagnostic tool is ideal for the rapid analysis of a high number of individuals and, thus, has potential applications in field contexts, where it could provide valuable baseline information for pest management programs.

Keywords: PCR-RFLP, *Bemisia tabaci*, *mtCOI* gene, molecular diagnostic, Sub-Saharan Africa group, MEAM1 species, Mediterranean species, pest management program.

1. Introduction

The whitefly *Bemisia tabaci* Gennadius is one of the most important pests worldwide, causing significant damage to food and ornamental crops directly by feeding on sap and, to an even greater extent, indirectly by transmitting many plant-pathogenic viruses (Jones 2003). *B. tabaci* displays considerable genetic and biological variability despite its morphological homogeneity, which has led to the conclusion that it is in fact a complex of morphologically indistinguishable 'cryptic' species (Perring 2001; De Barro et al. 2011). The genetic complexity of *B. tabaci* has mainly been investigated using mitochondrial cytochrome oxidase gene (*mtCO1*) sequence data (Frohlich et al. 1999; Boykin et al. 2007). This has led to the identification of several genetic groups known as biotypes, but the use of the term 'biotype' is now subjected to debate. The taxonomy and systematics of *B. tabaci* have not yet been completely elucidated, but recently it was proposed that a value of 3.5% *mtCO1* sequence divergence should be used to distinguish 28 putative cryptic species within the *B. tabaci* complex (Dinsdale et al. 2010; Hu et al. 2011; see De Barro et al. 2011 and Liu et al. 2012 for review). Those species fall into 11 major groups (> 11% divergence), which include those previously described as biotypes. In this study, we have adopted the nomenclature proposed by Dinsdale et al. (2010), but we have also included the biotype designation so as to make it easier to relate our study to the previously published literature.

Among the 28 putative species, two stand out as being the most predominant and devastating worldwide, the Middle East - Asia Minor species 1 (commonly known as biotype B; referred to hereafter as MEAM1) and the Mediterranean MED species (which includes the Q, J, L and ASL biotypes). These two species belong to the same Africa/Middle East/Asia Minor major group of *B. tabaci* identified by Dinsdale et al. (2010), which also includes MEAM2 and Indian Ocean IO species (formerly known as the MS biotype). The MED and

MEAM1 species have colonized large areas of most continents as a result of introduction/invasion processes (Bedford et al. 1994; Liu et al. 2007; De Barro and Ahmed 2011). Their invasive ability is mainly attributable to their having a broad spectrum of host plants, better reproductive and competitive potential, and greater insecticide resistance. In the Mediterranean basin, the MED species predominates. In some areas, it coexists locally with MEAM1. The MED species is common in North Africa, and has spread, probably recently, into West Central Africa and South Africa, where it coexists with the Sub-Saharan Africa indigenous species (also known as the AnSL biotypes; referred to hereafter as SSAF species) (Gueguen et al. 2010; Esterhuizen et al. 2013).

The MED species appears to be genetically diverse not only because it includes the Q, J, L, and Sub-Saharan Africa Silverleaf ASL biotypes (De Barro et al. 2011), but also because high genetic variability has been observed within the Q group, leading to the recognition of several cytotypes on the basis of *mtCO1* gene sequencing and symbiotic bacterial infection (Gueguen et al. 2010; Chu et al. 2012). Chu et al. (2012) recognized five subclades, designated as Q1–Q5, four of which correspond to the previously known Q1, Q2, Q3 and ASL genetic groups (Gueguen et al. 2010). These genetic groups display some particular features. First, they have different geographical and host plant ranges. For example, Q1 and Q2 have been sampled at a large geographical scale on a wide spectrum of host plants, whereas Q3 has only been observed in Western Africa on *Lantana camara* and tobacco (Gnankiné et al. 2012). The Q subclades also differ with regard to the composition of the bacterial symbiotic communities they harbor (Gueguen et al. 2010; Gnankiné et al. 2012). Some of these bacteria are known to induce drastic phenotypic change in *B. tabaci*, conferring features such as a fitness benefit (Kontseladov et al. 2008; Himler et al. 2011). Finally, they also differ as regards the

frequencies of alleles resistant to pyrethroids and organo-phosphates (Mouton et al. unpublished data).

The spread of the MED and MEAM1 species worldwide has had a considerable impact on agriculture (for review, see Oetting and Buntin 1996), and many studies have focused on identifying the mechanisms underlying their invasiveness and the best methods of control. This requires monitoring *B. tabaci* populations to track the evolution of the species composition in the invaded areas. Gene sequencing is costly and time-consuming, therefore, other effective diagnostic tools need to be developed in order to perform epidemiological studies involving hundreds of individuals. One of the various PCR-based methods, PCR-RFLP, consists of digestion with restriction enzymes after a polymerase chain reaction, and it provides a simple, fast and effective way to detect some of the variations in DNA sequences without sequencing. This technique has already been developed for use in *B. tabaci*, mainly to distinguish MEAM1, MED and non-MEAM1, non-MED species (Bosco et al. 2006; Tsagkarakou et al. 2007; Vassiliou et al. 2008; Ma et al. 2009; Shatters et al. 2009). Moreover, other PCR-RFLP protocols have been proposed to detect genetic variability in the Mediterranean basin, but these do not identify all the genetic diversity known to exist in the Africa/Middle East/Asia Minor group (Sartor et al. 2008; Chu et al. 2012). In this study, we describe a PCR-RFLP method that can be used to identify all the putative species that belong to the major groups (MED, MEAM1, MEAM2, Indian Ocean IO species), and also to recognize within-species variations. We also considered the Sub-Saharan Africa group, which is composed of several Sub-Saharan African species (SSAF1–5 species), which are sympatric with the MED species in some localities (Berry et al. 2004; Gueguen et al. 2010; Gnankiné et al. 2012; Esterhuizen et al. 2013).

2. Materials and methods

2.1. Phylogenetic analyses

We performed a phylogenetic analysis to determine the genetic diversity within *B. tabaci* populations in the two major groups under consideration in this study, *i.e.*, the Africa/Middle East/Asia Minor and the Sub-Saharan Africa clades. All the *mtCOI* sequences of *B. tabaci* available in Genbank for these two groups were analyzed, and only the sequences of around 657 bp with no undefined nucleotides, and no gaps or indels, were used. This gave us 143 sequences originating from samples collected worldwide (Table 1). Multiple sequence alignment was carried out using MUSCLE software (Edgar 2004) implemented in CLC DNA Workbench (CLC bio). Only non-redundant sequences were retained to construct the tree; this gave us 25 haplotypes (for each haplotype, the sequence used for the analysis is shown in bold type in Table 1). Phylogenetic analyses were performed using maximum likelihood and Bayesian inferences. The appropriate model of evolution selected with jModeltest v0.1.1 (Posada 2008) was *GTR+I+G* for both methods. ML analyses were performed with Phyml v 3.0 (100 bootstrap replicates) (Guindon et al. 2010). Bayesian analyses were done using MrBayes v 3.1.2 with 500,000 generations (Ronquist and Huelsenbeck 2003). Results were plotted using Figtree v1.3.1.

2.2. In-silico selection of restriction enzymes

Restriction enzymes were selected by *in silico* analyses of the 25 haplotypes using the CLC DNA Workbench 6.0 (CLC Bio) program. CLC sequence viewer was used to predict restrictions sites on a 657-bp fragment of the *mtCOI* gene sequence with 50 restriction enzymes.

2.3. PCR-RFLP on field samples

The PCR-RFLP tool developed in this study was tested on almost 1,100 *B. tabaci* individuals originating from France, Spain, Israel, Greece, Togo, Benin and Burkina Faso, and collected from various host plants (Table 2). DNA was extracted using the procedure of Gueguen et al. (2010). Briefly, individual insects were ground in 25 µL of extraction buffer containing 50 mM KCl, 10 mM Tris-base pH 8.0, 0.45% Nonidet P-40, 0.45% Tween 20 and 500 mg/mL proteinase K. Samples were incubated at 65 °C for 3 h and then at 100 °C for 15 min. A 35-µL aliquot of pure water was added to this extract, which was then stored at -20 °C until use. An 819-bp fragment of *mtCOI* was amplified with the primers C1-J-2195 and L2-N-3014 (Simon et al. 1994). PCR amplifications were performed in a final volume of 25 µL containing 200 µM dNTPs, 200 nM of each primer, 1.5 mM of $MgCl_2$, 0.5 U of *Taq* polymerase (EuroblueTaq, Eurobio, France) and 2 µL of DNA template. The cycling profile consisted of an initial denaturing phase at 95 °C for 2 min, followed by 35 cycles consisting of: 94 °C for 1 min (denaturing), 50 °C for 1 min (annealing) and 72 °C for 1 min (extension) followed by a final extension phase at 72 °C for 10 min. PCR products (10 µL) were digested with XapI, and another 10 µL was digested with BfmI (10 UI) at 37 °C for 3 h. The products obtained were then separated by electrophoresis on a 2% agarose gel at 100 V for 1 h and visualized by ethidium bromide staining. In parallel, PCR products were directly sequenced from 120 individuals.

3. Results

3.1. Phylogenetic structure

The phylogenetic tree obtained with the 25 selected haplotypes is consistent with the most recent *B. tabaci* phylogenies available in the literature (Dinsdale et al. 2010; De Barro et al. 2011). Since the ML and the Bayesian analyses led to substantially the same topology, we present only the ML analysis (Fig. 1). Genetic variability was observed within the major groups and species recognized by De Barro et al. (2011). Fifteen haplotypes were identified in the Africa/Middle East/Asia Minor group, and seven in the Sub-Saharan Africa group. Genetic diversity was also found within species, with up to nine haplotypes in the MED species and four in MEAM1. Polymorphism was lower in the other species analyzed (five haplotypes for SSAF5, three for SSAF1, two for SSAF2 and one for MEAM2, IO, SSAF3 and SSAF4), but this may have been due to the limited number of sequences available in Genbank for these species.

3.2. In silico PCR-RFLP

Two restriction enzymes were selected after *in-silico* analysis on the basis of their capacity to discriminate between the Africa/Middle East/Asia Minor and Sub-Saharan Africa major groups, and their ability to discriminate within these groups between the putative species described by Dinsdale et al. (2010) and De Barro et al. (2011). Ten and nine profile types were found for *XapI* and *BfmI*, respectively (Fig. 2). Both enzymes were able reliably to distinguish between the two major groups. For *XapI*, we found profile types #1, 2, 5, 8, 9 and 10 for the Africa/Middle East/Asia Minor group, and profile types #3, 4, 6 and 7 for the Sub-Saharan Africa major group. For *BfmI*, profiles #1, 2, 4, 5, 6, 8, 9 correspond to the

Africa/Middle East/Asia Minor group, while profiles #3 and 7 characterized the Sub-Saharan Africa major group (Fig. 1, Table 1). Seventeen profiles were obtained when simultaneous DNA digestion by both enzymes was performed: 12 for the Africa/Middle East/Asia Minor group and 7 for the Sub-Saharan Africa group (Table 1). Within the Sub-Saharan Africa group, we were unable to distinguish all the SSAF species. In contrast, within the Africa/Middle East/Asia Minor group, PCR-RFLP profiles distinguished all the putative species: profiles 1, 2 and 3 were particular to the MEAM1 species; profiles 5 to 11 were specific to the MED species, and profiles 4 and 12 characterized the MEAM2 and IO species, respectively. In summary, *in-silico* analysis suggests that *XapI* and *BfmI* acting simultaneously offer good potential identification of all four putative species in the Africa/Middle East/Asia Minor group, including the ability to detect within-species diversity, since 12 profiles were obtained for 15 known haplotypes.

3.3. PCR-RFLP on field samples

The *in-silico* predictions were checked on field samples belonging to the Africa/Middle East/Asia Minor (MEAM1 and MED species) and the Sub-Saharan Africa (SSAF1 and SSAF2 species) groups. We performed both PCR-RFLP and *mtCOI* gene sequencing on 120 *B. tabaci* individuals collected worldwide from several plants (Table 2). For another 959 individuals, the identification was done using the PCR-RFLP method only, in order to check the reliability of the method. Digestion with *XapI* and *BfmI* generated six and four distinct restriction patterns in our samples, respectively, differing by the number and/or size of the fragments, making it possible to identify the MEAM1, MED and SSAF species (Fig. 3) as predicted by the *in-silico* analysis (Table 1). Moreover, restriction profiles differentiated haplotypes within the MED species, making it possible to recognize the commonly known

ASL, Q1, Q2, Q3 genetic groups previously described as different biotypes or cytotypes, which harbor different bacterial endosymbionts. Fragments of less than 50 bp were not detected, because they were too small to be visualized by electrophoresis in a routine agarose gel assay. Moreover, the size of the bands observed for some restriction profiles deviated slightly from predictions; this was because the *in-silico* analysis was performed on 657 bp, which is only a part of the 867-bp amplicon obtained with the primers used to amplify the *mtCOI* gene in *B. tabaci* (C1-J-2195 and L2-N-3014; Simon et al. 1994).

For all 120 individuals in which biotype identification was carried out using both standard *mtCOI* gene sequencing and PCR-RFLP assay, the same results were obtained by both methods (Table 2). Moreover, we did not detect any unexpected restriction profiles for the 959 other individuals screened by PCR-RFLP only. This implies there was no other sequence variation at the *XapI* and *BfmI* restriction sites than those detected by the *in silico* analysis.

4. Discussion

In this paper a simple diagnostic tool, based on a PCR-RFLP method on the *mtCO1* gene was used:

1) To reliably distinguish between the two major groups of *B. tabaci* that coexist in sympatry in some places in the Mediterranean basin and Africa (Gueguen et al. 2010; Gnankiné et al. 2012; Esterhuizen et al. 2013): the Africa/Middle East/Asia Minor group, which includes the two most invasive members (B and Q biotypes), and the Sub-Saharan Africa group, which is regarded as the basal clade of *B. tabaci.*

2) To detect all the putative species defined in the Africa/Middle East/Asia Minor group by De Barro et al. (2011) and Dinsdale et al. (2010). These species include MEAM1 (formerly referred to as biotype B) and MED (biotype Q), which are widespread and considered to be the ones that cause the most damage (Oetting and Buntin 1996). In many places, displacement of MEAM1 by MED has been observed (Horowitz et al. 2005; Crowder et al. 2010), and the mechanism(s) underlying this phenomenon have been investigated for many years; this requires monitoring *B. tabaci* populations to track the evolution of the species composition in these areas. One explanation for this displacement could be the fact that these two species differ in their susceptibility towards various insecticides. Indeed, it has been demonstrated that the use of neonicotinoids or pyriproxyfen positively selects for the MED species, which exhibits greater resistance to these insecticides than the MEAM1 species (Horowitz et al. 2005).

Genetic diversity at the mitochondrial level has also been detected at a finer scale, *i.e.*, within species. This PCR-RFLP protocol makes it possible to identify entities previously recognized as the Q1, Q2, Q3 and ASL genetic groups on the basis of *mtCO1* gene sequencing. While it is still not completely clear whether these groups are of any biological significance, some arguments suggest that this distinction should be taken into account. First, these haplotypes harbour particular symbiont communities that can potentially modify a number of biological traits in their host (Chiel et al. 2007; Gueguen et al. 2010; Gnankine et al. 2012). Second, all the genetic groups of MED species are structured geographically (Gueguen et al. 2010; Gnankiné et al. 2012), so that tracking them provides a way of identifying the invasion routes of *B. tabaci*. Lastly, they are associated with biological features, such as host plant use (Gnankiné et al. 2012), and the frequency of insecticide-resistant alleles (Mouton et al., unpublished data). This further suggests that the mitochondrial diversity could also be associated with nuclear variation and possible genetic isolation. This last point is important because species definition by Dinsdale et al. (2010) is based on *mtCOI* divergence, which means that some so-far unidentified, genetically isolated entities may exist at a finer phylogenetic scale. In a recent study, Chu et al. (2012) found five haplotypes within the MED species by *mtCO1* sequencing. However, the PCR-RFLP method they developed for the *mtCOI* gene using the restriction enzyme *VspI* only distinguished one haplotype, which corresponds to the commonly known Q1 biotype, but the method was unable to detect the other four. The tool we developed here can be used to identify four out of the five *mtCOI* haplotypes described in the MED species. It can thus detect diversity at a very fine scale, which may make it very useful in population studies and may have major implications for pest management programs.

Levels of resistance and resistance mutation frequencies to insecticides differ between *B. tabaci* species (Horowitz et al. 2005; Wang et al. 2010; Alon et al. 2006, 2008) but also within species as has been observed within the MED species (Mouton pers. com.) Therefore, to be effective, strategies developed to control this pest must differ according to the local *B. tabaci* composition and the chemical classes of insecticides used. Monitoring the evolution of genetic diversity is, thus, clearly required for effective control strategies of this pest. The tool developed here has already been successfully used for describing the diversity of *B. tabaci* genetic groups in western Africa (Gnankiné et al. 2012; Gnankiné et al. 2013; Mouton, pers. com.) and could be useful to monitor the dynamics of *B. tabaci* populations in relation to insecticide resistance in countries, like west African countries, where insecticides are widely used and lead to high levels of resistance (Houndété et al. 2010).

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References

Alon M, Alon F, Nauen R, Morin S. 2008. Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an ace1-type acetylcholinesterase and overexpression of carboxylesterase. Insect Biochem Mol Biol 38: 940-949.

Alon M, Benting J, Lueke B, Ponge T, Alon F, Morin S. 2006. Multiple origins of pyrethroid resistance in sympatric biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Insect Biochem Mol Biol 36: 71-79.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Ann Appl Biol 125: 311-325.

Berry SD, Fondong VN, Rey MEC, Rogan D, Fauquet C., Brown JK. 2004. Molecular evidence for five distinct *Bemisia tabaci* (Homoptera; Aleyrodidae) geographic haplotypes associated with cassava plants in sub-Saharan Africa. Ann Entomol Soc Am 97: 852-859.

Bosco D, Loria A, Sartor C, Cenis JL. 2006. PCR-RFLP Identification of *Bemisia tabaci* biotypes in the Mediterranean Basin. Phytoparasitica 34: 243–251.

Boykin LM, Shatters RG, Rosell RC, McKenzie CL, Bagnall RA, De Barro P, Frohlich DR. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. Mol Phyl Evol 44: 1306-1319.

Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, Ghanim M. 2007. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. Bull Entomol Res 97: 407-413.

Chu D, Hu X, Gao C, Zhao H, Nichols RL, Li X. 2012. Use of mitochondrial cytochrome oxidase I polymerase chain reaction-restriction fragment length polymorphism for

identifying subclades of *Bemisia tabaci* Mediterranean group. J Econ Entomol 105: 242-251.

Crowder DW, Horowitz R, De Barro PJ, Liu SS, Showalter AM, Kontsedalov S, Khasdan V, Shargal A, Liu J, Carriere Y. 2010. Mating behaviour, life history and adaptation to insecticides determine species exclusion between whiteflies. J Anim Ecol 79: 563-570.

De Barro P, Ahmed M. 2011. Genetic networking of the *Bemisia tabaci* cryptic species complex reveals pattern of biological invasions. PLoS ONE 6: e25579.

De Barro PJ, Liu SS, Boykin LM, Dinsdale B. 2011. *Bemisia tabaci*: a statement of species status. Ann Rev Entomol 56: 1-19.

Dinsdale A, Cook L, Riginos C, Buckley YM, De Barro P. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidae: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Ann Entomol Soc Am 103: 196-208.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5: 113.

Esterhuizen LL, Mabasa KG, van Heerden SW, Czosnek H, Brown JK, van Heerden H, Rey MEC. 2013. Genetic identification of members of the *Bemisia tabaci* cryptic species complex from South Africa reveals native and introduced haplotypes. J Appl Entomol 137: 122-135.

Frohlich DR, Torres-Jerez I, Bedford ID, Markham PG, Brown JK. 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol Ecol 8: 1683-1691.

Gnankiné O*, Mouton L*, Henri H, Terraz G, Houndeté T, Martin T, Vavre F, Fleury F. 2012. Distribution of the *Bemisia tabaci* biotypes (Homoptera: Aleyrodidae) and their

associated symbiotic bacteria on host plants in Western Africa. (*1st co-authors). Insect Cons Div, DOI: 10.1111/j.1752-4598.2012.00206.x

Gnankiné O, Mouton L, Savadogo A, Martin T, Sanon A, Vavre F, Fleury F. 2013. Biotype status and resistance to neonicotinoid and carbosulfan in *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Burkina Faso, West Africa. Int J Pest Manage, DOI: 10.1080/09670874.2013.771806.

Gueguen G, Vavre F, Gnankine O, Peterschmitt M, Charif D, Chiel E, Gottlieb Y, Ghanim M, Zchori-Fein E, Fleury F. 2010. Endosymbiont metacommunities, *mtDNA* diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. Mol Ecol 19: 4365-4378.

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. System Biol 59: 307-321.

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Arch Insect Biochem Physiol 58: 216-225.

Houndété TA, Kétoh GK, Hema OSA, Brévaukt T, Glitho IA, Martin T. 2010. Insecticide resistance in field populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in West Africa. Pest Manag Sci 11: 1181-1185.

Hu J, De Barro P, Zhao H, Wang J, Nardi F, Liu S-S. 2011. An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. Plos One 6: e16061.

Jones DR. 2003. Plant viruses transmitted by whiteflies. Eur J Plant Pathol 109: 195-219.

361 Liu SS, Colvin J, De Barro PJ. 2012. Species concepts as applied to the whitefly *Bemisia*
362 *tabaci* systematics: how many species are there? J Int Agr 11: 176-186.
363 Liu SS, De Barro PJ, Xu J, Luan JB, Zang LS, Ruan YM, Wan FH. 2007. Asymmetric mating
364 interactions drive widespread invasion and displacement in a whitefly. Science 318: 1769-
365 1772.
366 Ma W-H, Li X-C, Lei C-L, Wang M, Degain BA, Nichols RL. 2009. Utility of mtCOI
367 polymerase chain reaction-restriction fragment length polymorphism in differentiating
368 between Q and B whitefly *Bemisia tabaci* biotypes. Insect Sci 16: 107-114.
369 Oetting RD, Buntin GD. 1996. Bemisia damage expression in commercial greenhouse
370 production. In: Gerling, D., Mayer, R.T. (Eds.), Bemisia: 1995 Taxonomy, Biology,
371 Damage, Control and Management. Intercept, UK, pp. 201-208.
372 Perring TM. 2001. The *Bemisia tabaci* species complex. Crop Prot 20: 725-737.
373 Posada D. 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol 25: 1253-1256.
374 Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed
375 models. Bioinformatics 19: 1572-1574.
376 Sartor C, Demichelis S, Cenis JL, Coulibaly AK, Bosco D. 2008. Genetic variability of
377 *Bemisia tabaci* in the Mediterranean and Sahel regions. Bull Insect 61: 161-162.
378 Shatters RG, Powell C, Boykin LM, Liansheng H, McKenzie CL. 2009. Improved DNA
379 barcoding method for *Bemisia tabaci* and related Aleyrodidae: development of universal
380 and *Bemisia tabaci* biotype-specific mitochondrial cytochrome c oxidase I polymerase
381 chain reaction primers. J Econ Entomol 102: 750-758.
382 Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and
383 phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR
384 primers. Ann Entomol Soc Am 87: 651-701.

385 Tsagkarakou A, Tsigenopoulos CS, Gorman K, Lagnel J, Bedford ID. 2007. Biotype status
386 and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in
387 Greece: mitochondrial DNA and microsatellites. Bull Entomol Res 97: 29-40.
388 Vassiliou VA, Jagge C, Grispou M, Pietrantonio PV, Tsagkarakou A. 2008. Biotype status of
389 *Bemisia tabaci* from various crops in Cyprus. Phytoparasiticia 36: 400-404.
390 Wang Z, Yan H, Yang Y, Wu Y. 2010. Biotype and insecticide resistance status of the
391 whitefly *Bemisia tabaci* from China. Pest Manag Sci 66: 1360-1366.
392

Figure legends

Figure 1: *mtCOI* gene-based phylogeny of the Africa/Middle East/Asia Minor and Sub-Saharan Africa major groups of *B. tabaci* using maximum likelihood (ML) analyses.

Bootstrap values are shown at the nodes for ML analysis, followed by the corresponding posterior probabilities obtained by Bayesian inferences. Sequences were retrieved from Genbank. *Bemisia afer* was used as the outgroup. The analysis was based on a 657-bp sequence. Biotypes and access numbers are indicated at each branch, as is the type of profile obtained with each enzyme (*XapI*/*BfmI*). Frames indicate the specific profiles that can be used to distinguish between the sequences (for example, profiles #10 and #2 obtained with XapI are specific to the MEAM1 species, whereas restriction with both enzymes is required to identify Q1, Q2, Q3, and L2).

Figure 2: *In-silico* restriction profiles obtained with *XapI* **(**A) and *BfmI* (B). The sizes of the bands corresponding to the ladder are indicated (bp).

Figure 3: PCR-RFLP assays with *XapI* (A) and *BfmI* (B).

The sizes of the different bands obtained, and the sizes (bp) of the bands corresponding to the ladder are indicated on the left and the right of the figure, respectively.